STUDY OF HYDROXYAPATITE AND HYDROXYAPATITE-CHITOSAN COMPOSITE COATINGS ON STAINLESS STEEL BY ELECTROPHORETIC DEPOSITION METHOD

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF

Bachelor in Technology

in

Biomedical Engineering

by

AJAY SAXENA (107BM003)

and

ABHINANDAN ROUT (107BM009)

Department of Biotechnology and Medical Engineering

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CERTIFICATE

This is to certify that the thesis entitled '**The study of hydroxyapatite and hydroxyapatite-chitosan composite coatings on stainless steel by electrophoretic deposition method**' submitted by **Ajay Saxena** and **Abhinandan Rout** in partial fulfilment of the requirements for the award of Bachelor of Technology from the Department of Biotechnology and Medical Engineering with specialization in "Biomedical Engineering" at National Institute of Technology, Rourkela (Deemed University) is an authentic work carried out by them under my supervision and guidance. To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/Institute for the award of any degree or diploma.

Place:

Date:

Supervisor

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Ajay Saxena Abhinandan Rout

Date:

Place:

ABSTRACT

Hydroxyapatite is a salt of calcium and phosphorus having the Ca/P ratio as 1.6. Hydroxyapatite is one of the major mineral components of the bone due to which it shows excellent biocompatibility. Even though it is biocompatible, hydroxyapatite cannot be used directly in load bearing structures because of its poor mechanical properties. An alternative approach to utilize the biocompatibility of hydroxyapatite is by using it as a coating material.

The biocompatibility of hydroxyapatite needs to be complemented by coating it on a substance having desirable mechanical properties. A suitable material for this purpose is medical grade stainless steel, which has been used to make prosthesis, bone fixation screws, guide wires etc. It has been shown that coating medical grade stainless steel (SS 316L) implants with hydroxyapatite leads to better biocompatibility. For the coating process various techniques can be used. Electrophoretic deposition is a procedure of mild nature not involving extreme temperatures and has the advantage of producing a stoichiometric, uniform coating of the desired thickness. In this study a practical approach to coating was undertaken and a HA and composite HA-Chitosan coating obtained by varying the processing parameters was studied.

A coating of HA and composite coating of stoichiometric hydroxyapatite and chitosan were produced on medical grade stainless steel (SS 316L). Industrially pure chitosan was bought for the coating purposes and hydroxyapatite nanoparticles were synthesized by a chemical reaction of calcium nitrate and ammonium phosphate. In the synthesis process of HA

nanoparticles the pH was controlled by the addition of ammonium hydroxide. The coating was carried out stainless steel as the cathode and graphite rod as the counter electrode, the distance between electrodes being 2 cm at a current density of 0.11 Ampere per metre square.

The SEM analysis showed that at a constant voltage the coating deposition increases with an increase in the coating time, whereas when the coating time is kept constant, the fineness of the coating increases with an increase in the voltage. From the XRD analysis it was confirmed that the phase purity of chitosan and HA is maintained even after the coating.

CONTENTS

CHAPTER 1

INTRODUCTION

A wide variety of materials are used for medical purposes. These include but are not limited to ceramic, glasses, metallic and polymeric biomaterials. Among these materials, metallic biomaterials find application where load bearing structures are required such as in orthopaedics and dentistry. The advantages of using metallic biomaterials include the relatively easy fabrication of both simple and complex shapes by the help of well-established fabrication techniques such as casting, forging, machining. The various biomaterials that are used include stainless steel, titanium, tantalum, nickel titanium alloys. Medical grade stainless steel (SS 316L) is widely used in fracture repair devices and joint replacement components. Its wide usage is due to the fact that it is a more economical alternative as compared to other metallic biomaterials. However 316L SS has a high susceptibility to corrosion and to overcome this shortcoming surface modification of stainless steel is done before its usage in the body. The common biocompatible coatings are of thermally grown oxides, electrolytically grown oxides and hydroxyapatite, the various processes used for the coatings are thermal exposure (poly crystalline and amorphous oxide), proprietary processes and electropolishing (in a phosphoric acid glycerine solution). HA coated SS 316L shows a positive shift in the OCP which is an indication of stable coating/insulation behaviour, along with an improvement in the pitting corrosion. The coating processes have the advantage of changing the properties of the surface of the material without affecting the properties of bulk. The alternative to coating process is the use of highly corrosion resistant alloy, but using highly corrosion resistant alloys will increase the cost of the implants making them economically infeasible. Along with the conventional techniques of thermal oxidation and electrochemical oxidation, advanced techniques plasma ion implantation, plasma source ion

implantation, laser melting, laser alloying, ion beam and physical vapour deposition have been widely applied to biomaterials. Electrophoretic deposition can be used to produce stoichiometric coating. It has the advantage of being able to produce uniform deposits with high microstructural homogeneity; it also provides adequate control of the deposit thickness. Electrophoretic deposition can be used to coat a wide range of shapes and 3D complex and porous structures.

Hydroxyapatite is the major mineral constituent of the bone and hence as a biomaterial hydroxyapatite shows good biocompatibility and bone adhesion. However hydroxyapatite suffers from poor mechanical properties and implants made entirely from Hydroxyapatite will suffer from a mechanical failure. To utilize the excellent biocompatibility of hydroxyapatite it can be used as a coating material for stainless steel, by which it will complement the mechanical strength of stainless steel. Such an implant made of stainless steel coated with hydroxyapatite can be used for bone fixation as it will show both a high strength and high biocompatibility. In the present study a coating of HA and a composite coating of hydroxyapatite chitosan was applied on stainless steel substrate by electrophoretic deposition method. Chitosan is a naturally occurring cationic polysaccharide. It is biocompatible, antimicrobial and biodegradable.

CHAPTER 2

LITERATURE SURVEY

2.1. Stainless steel

With improved medical facilities worldwide, more and more people are living to advanced ages in today's world. As a result, the chances of body parts (especially those subjected to stress and pressure on a regular basis like bones and joints) wearing out and stopping functioning are much greater over this extended period of time. So, implants are being increasingly used in today's world to replace the functioning of bones and joints.

Since these parts are subjected to various stresses, the implants need to be strong enough to absorb all of the stress that will be inflicted upon them by the body. The implants should have good mechanical properties, high corrosion resistance, be cheaply available, have good fatigue life, and undergo no wear and tear while functioning. Generally, metals and metallic alloys have been used as implant materials because they fulfil most of these requirements. Stainless steel (technically 316L stainless steel) has been increasingly used as implant materials because of its similarities to human bone in terms of young's modulus of elasticity, hardness and other mechanical properties. It is considered as one of the attractive metallic materials for biomedical applications also due to its mechanical properties, biocompatibility, and corrosion resistance. This material is a popular metal for use as acetabular cup (one half of an artificial hip joint) applications [14].

2.2. Hydroxyapatite

Hydroxyapatite (HA) is a bioactive ceramic material with high bioaffinity, biocompatibility and osseoconductivity which is the main constituent of bones and teeth. Natural HA has the advantage that it inherits some properties of the raw material such as composition and

structure. Properties of HA have found useful application in low-load bearing porous implants and coatings of metallic implants. Bioactivity and acting as a template for forming and growing of the surrounding bone tissues make HA an excellent choice for coating of the metallic implants. After implantation of prostheses, a close surface contact between the metallic prosthesis and the surrounding bone tissue is needed for subsequent bone ingrowths. Coating of the metallic implants with bioactive HA leads to a rapid bonding between hydroxyapatite and surrounding bone tissue. Application of HA as a coating of the metallic implants combines the strength and toughness of the substrate with bioactive characteristic of HA which can induce the surrounding bone tissue ingrowths and future formation of chemical bonding. Further, the presence of HA coating can improve corrosion resistance of the coated implant in human body which can reduce the metallic ion release and also promotes fixation via chemical bonding [15].

2.3. Chitosan

Chitosan is a cationic polysaccharide and one of the most promising natural biopolymers for tissue engineering, biocompatible coatings and drug delivery. Due to its unique properties including biodegradability, biocompatibility, non-toxicity and bio-functionality, Chitosan has attracted much attention for a wide variety of applications ranging from skin, bone, cartilage and vascular grafts to substrates for cell culture. Previous studies have shown the feasibility of cationic electrophoretic deposition (EPD) of chitosan. The deposition mechanism is related to the pH variation caused by electrochemical decomposition of water. A recent progress has been the chitosan-mediated electrosynthesis of composites based on bioactive glass, hydroxyapatite (HA), bioactive glass–HA, HA–wollastonite, HA–carbon nanotubes and HA–CaSiO3. More recently, Mayer et al. have used electrodeposition of chitosan on gold wires to

fabricate a biosensing platform, showing the high potential of the method to develop novel chitosan based devices. It is evident that knowledge of the electromigration of chitosan molecules is necessary in order to control the deposition rate and to achieve flexibility in microstructural manipulation of chitosan coatings. Aider et al. have shown that the electromigration of chitosan D-glucosamine and oligomers in aqueous solutions depends on the pH. To the best knowledge of the authors, the growth rate of chitosan film macromolecules as a function of pH and biopolymer concentration has never been reported. Understanding the electrophoretic mobility and deposition kinetics of chitosan will not only be useful for controlling the deposition of neat chitosan films but also to describe the interaction between the polymer and ceramic particles during co-deposition in order to control the EPD process of inorganic–organic composite coatings, which are of high relevance for biomedical applications [16].

2.4. Techniques of coating

There are several coating methods used for surface modifications of materials. The following techniques are a few of them used for applying coatings on metals:

- Electroplating – Electroplating is a process of coating a deposition on a cathode part immersed into an electrolyte solution, where the anode is made of the depositing material, which is dissolved into the solution in the form of the metal ions, that travel through the solution and deposit on the cathode surface.

- Electroless plating - The process of deposition of metal ions from electrolyte solution onto the substrate when no electric current is involved and the plating is a result of

chemical reactions occurring on the surface of the substrate.

- Conversion coating - The process in which the coating is formed as a result of chemical or electrochemical reaction on the substrate surface. These are non-metallic coatings obtained on the metal surface in the form of compounds of the substrate metals.

- Hot dipping - Immersing the part into a molten metal followed by removal of the substrate from the metal bath, which results in formation of the metal coating on the substrate surface.

- Physical Vapour Deposition (PVD) - the process involving vaporization of the coating material in vacuum, transportation of the vapour to the substrate and condensation of the vapour on the substrate surface.

- Chemical Vapour Deposition (CVD) – The process, in which the coating is formed on the hot substrate surface placed in an atmosphere of a mixture of gases, as a result of chemical reaction or decomposition of the gases on the substrate material.

- Thermal spraying – Deposition of the atomized at high temperature metal, delivered to the substrate surface in a high velocity gas stream

2.5 Electrophoretic Deposition

Electrophoretic deposition is a conventional technique. But it is used vastly due to its certain advantages over other coating techniques like that of low cost, low energy requirement, capability to handle complex geometry, simple scale-up with easily maintainable equipment and good chemical stability. One of its important advantages is that a very large number of pure metals, alloys, composites and ceramics can be electrodeposited with grain size less than 100 nm. Metals, alloys and biopolymers can also be deposited by this process. Multilayer deposition is possible in this process. Materials can be coated for use in industrial applications such as coatings of engine cylinders, high pressure valves, musical instruments, car accessories, small aircraft microelectronics, aerospace, medical devices, marine, agriculture and nuclear fields [17].

2.5.1 Mechanism of Electrophoretic Deposition

Electrophoretic deposition is a process by which charged ions in the solution are attracted and deposited onto an electrode of the opposite charge by the application of an electric field. Electrophoretic deposition is essentially a two-step process. In the first step the charged particles migrate under the electric field to the electrode having the opposite charge. In the second step, the particles deposit on the electrode forming a relatively dense, compact and homogenous film. There can be two types of electrophoretic deposition processes on the basis of the electrode on which the coating is produced. When the particles are positively charged the deposition happens on the cathode the process is called cathodic electrophoretic deposition whereas when the particles are negatively charged and the deposition occurs on the anode the process is known as anodic electrophoretic deposition. The process leads to creation of stoichiometric films whose composition depends on the stoichiometry of the

powder used. The principal driving force for electrophoretic deposition is the charge on the particle and its electrophoretic mobility in the solvent under the presence of the applied electric field. According to Sarkar and Nicholson, particle/electrode reactions are not involved in EPD and ceramic particles do not lose their charge on being deposited [18].

Fig 1: Electrophoretic deposition apparatus

2.5.2 Effect of processing parameters on deposition

The parameters related to the EPD can be categorized into solution related and process related parameters. The solution related parameters are particle size, dielectric constant of the liquid, conductivity and viscosity of the suspension, zeta potential and stability of the suspension. The process related parameters are deposition time, applied voltage, concentration of the solid in suspension and conductivity of the substrate,

- **Effect of deposition time**

Basu et al have found that for a fixed electric field when the deposition time increases the deposition rate decreases, similar observations were made by Chen and Liu. This is attributed to the decrease in the electric field influencing electrophoresis due to the formation of an insulating layer of ceramic particles on the electrode surface (Zhitomirsky and L Gal).

- **Effect of Applied Voltage**

It is observed that the deposit increases with an increase in voltage. When stronger electric fields are used, the deposition rate increases but the quality of the deposit gets affected. Basu et al found that more uniform films are deposited at moderate applied fields (25-100 V/cm) whereas film quality deteriorates if relatively higher applied fields are used. The stronger electric fields cause turbulence in the medium which affects the developing coating; also the nature of the coating that is produced depends on the accumulation rate which influences the packing behaviour of the particles in the coating. In case of a stronger electric field, the accumulation rate is higher and the particles move faster; so they don't have time to sit in their positions in close packed structure. The lateral motion of the particles is restricted on the surface of the already deposited layer as the higher field exerts more pressure on particle flux and movement.

- **Effect of concentration of solid in suspension**

The rate at which the particles deposit on the electrodes depends on their electrophoretic mobilities; it becomes an important factor when the volume fraction of the solids is low. When the volume fraction of the solids is high, they deposit at an equal rate. However, when the volume fraction of the solids is low the particles deposit at rate proportional to their individual electrophoretic mobility (Vandeperre, Biest and Clegg).

CHAPTER 3

EXPERIMENTAL PROCEDURE

3.1 Sample and solution preparation for HA coating

1. A steel rod having diameter 1 cm was cut into cylindrical samples of length 1 cm.
2. Samples were polished by belt grinder and emery paper of standard 1/0, 2/0, 3/0 and 4/0 successively. After this, cloth polishing was done by using aluminium oxide. On completion of cloth polishing diamond polishing was done.
3. HA obtained from chemical synthesis was dissolved in ethanol with the help of magnetic stirrer and 150 ml of this solution was taken.

3.2 Sample and solution preparation for HA-Chitosan composite coating

1. A steel rod having diameter 1 cm was cut into cylindrical samples of length 1 cm.
2. Sample was polished by belt grinder and emery paper of standard 1/0, 2/0, 3/0 and 4/0 successively. After this, cloth polishing was done by using aluminium oxide. On completion of cloth polishing diamond polishing was done.
3. HA obtained from chemical synthesis was dissolved in ethanol with the help of magnetic stirrer and 150 ml of this solution was taken.
4. A solution was formed by dissolving 0.4 gm chitosan in 500 ml acetic acid and water solution, where the pH was maintained between 3.5 and 3.9 and 150 ml of this solution was taken.
5. 300 ml of the final solution was prepared by mixing the solutions obtained in the above two steps.

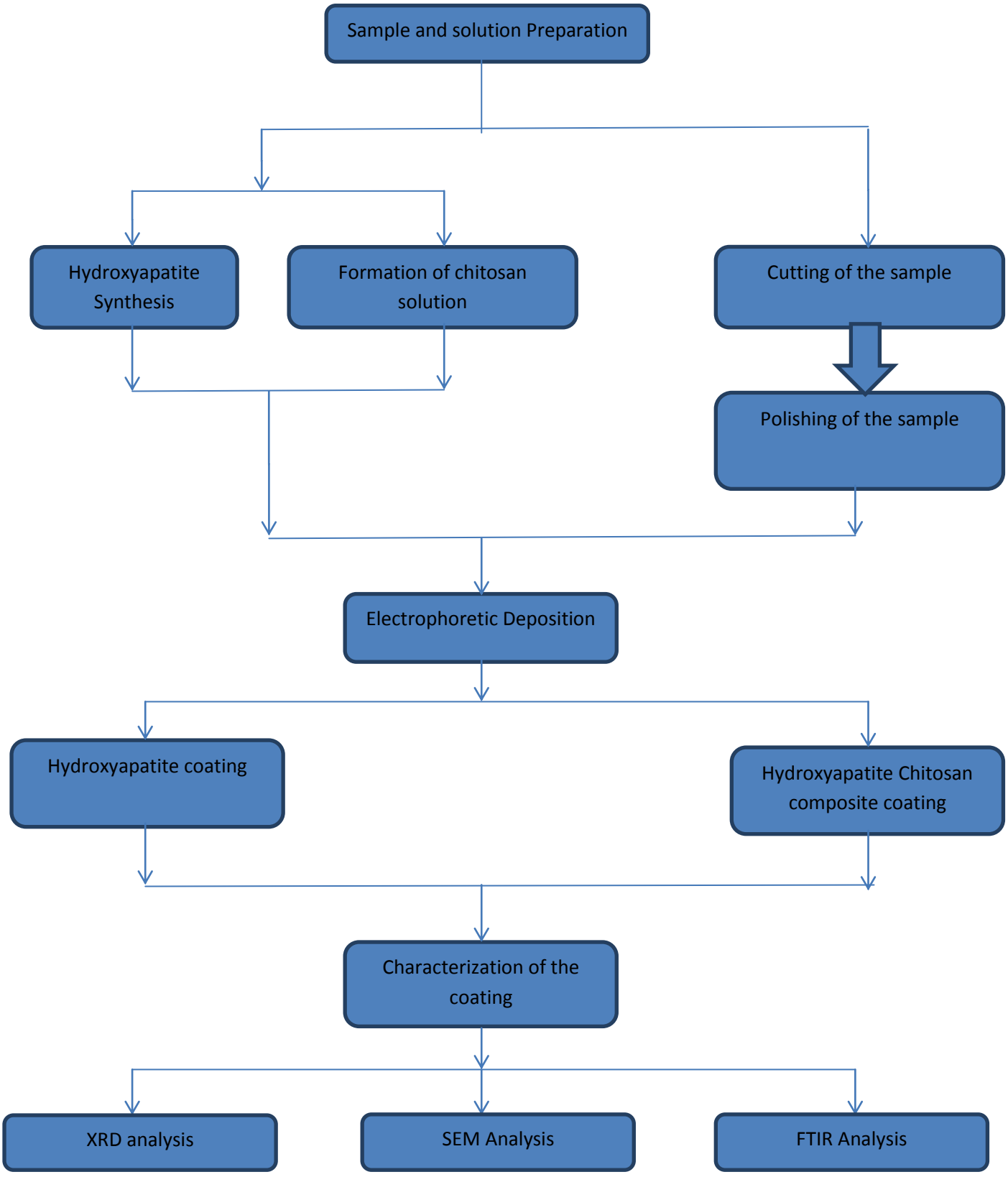
3.3 Experimental procedure for electrophoretic deposition

1. The electrodes were washed with acetone and dried.
2. The deposition was carried out at room temperature from 150 ml of the solution (as per the coating desired) in 250 ml beaker. The solution was stirred using a magnetic stirrer.
3. A graphite rod was used as an anode and the sample was used at cathode.
4. The distance between the electrodes was 2 cm.
5. Deposition was carried out on a substrate surface area of 0.785 cm^2.
6. HA and HA-chitosan composite was deposited on the stainless steel by electrophoretic deposition.
7. For deposition of HA, voltages were varied from 15 V, 20V, 25 V for time periods of 10 min, 15 min, 20 min.
8. For deposition of HA-chitosan composite coatings voltages were varied from 20 V, 25 V, 30 V for time periods of 15 min, 20 min, 30 min.

3.4 Characterization

The SEM analysis of the sample was carried out on JEOL JSM 6480 lv, to study the microstructure of the coating. The phase purity was tested by X Ray diffraction on Philips Xpert PANalytical XRD machine.

3.4 FLOWCHART DEPICTING THE EXPERIMENTAL PROCEDURE



CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This project is aimed at a detailed investigation of the electrophoretic deposition of HA and HA-chitosan layer on 316L stainless steel. The surface characterization using scanning electron microscope (SEM) and X-ray diffraction (XRD) of the coated layer have been done.

4.2 Electrophoretic deposition of hydroxyapatite (HA) on 316L Stainless steel

4.2.1 Characteristics of the HA layer on 316L Stainless steel

4.2.1.1 Microstructural analysis

Figure 4.1 shows the effectiveness of the HA coating on 316L SS with time. From the figures 4.1 (a), (b) and (c), it is observed that HA is unformly deposited on the surface.when seen under low magnification. But in case of Fig 4.1 (c), it is observed that microcracks are formed on the coated surface. This is due to formation of a thicker layer which undergoes rearrangement upon contraction leading to separation. If the layer were thinner, formation of cracks would be difficult because the metallic substrate would have adhered better to the thinner HA layer.

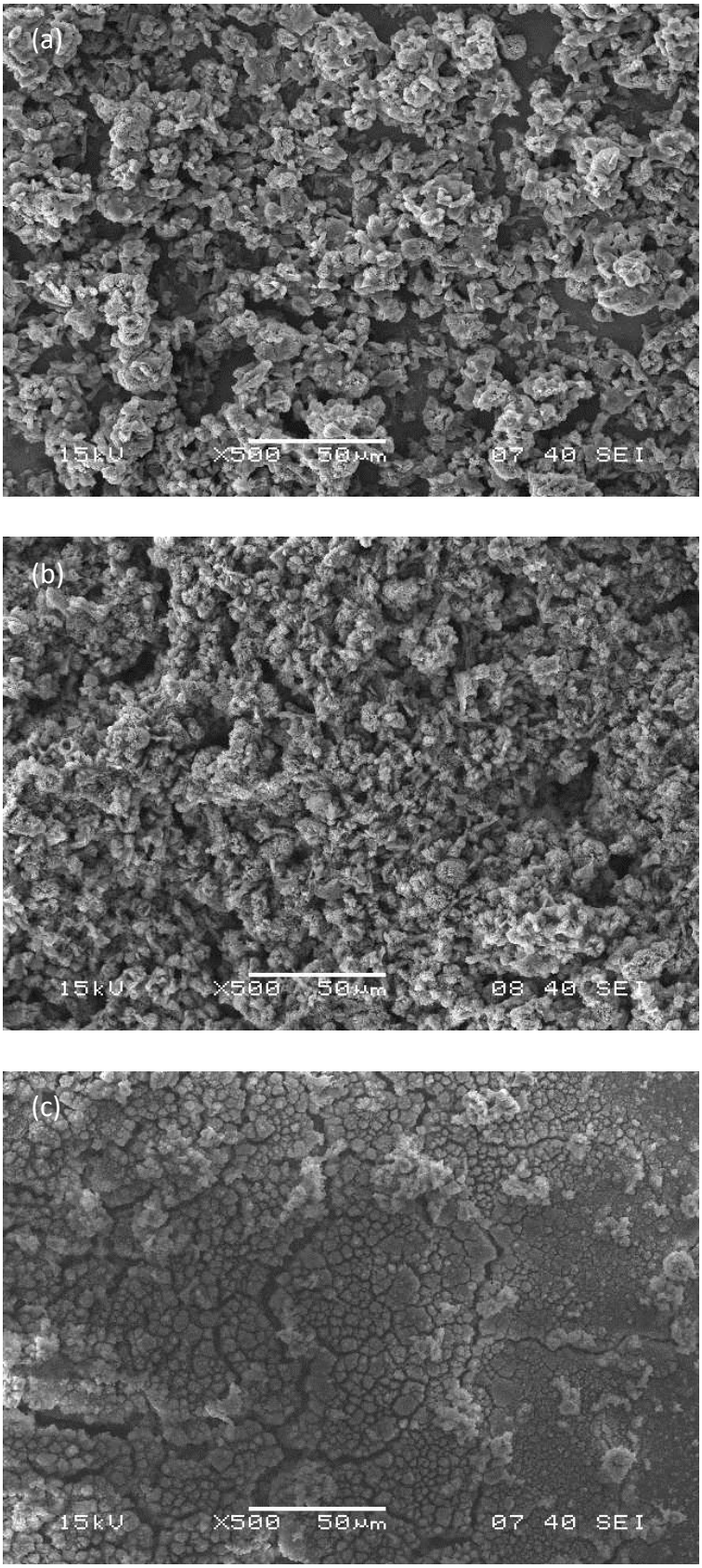


Fig 4.1: Scanning electron micrograph (500x magnification) of electrophoretically deposited HA on 316L stainless steel at (a) 30 V - 10 mins (b) 30 V - 15 mins (c) 30 V - 20 mins

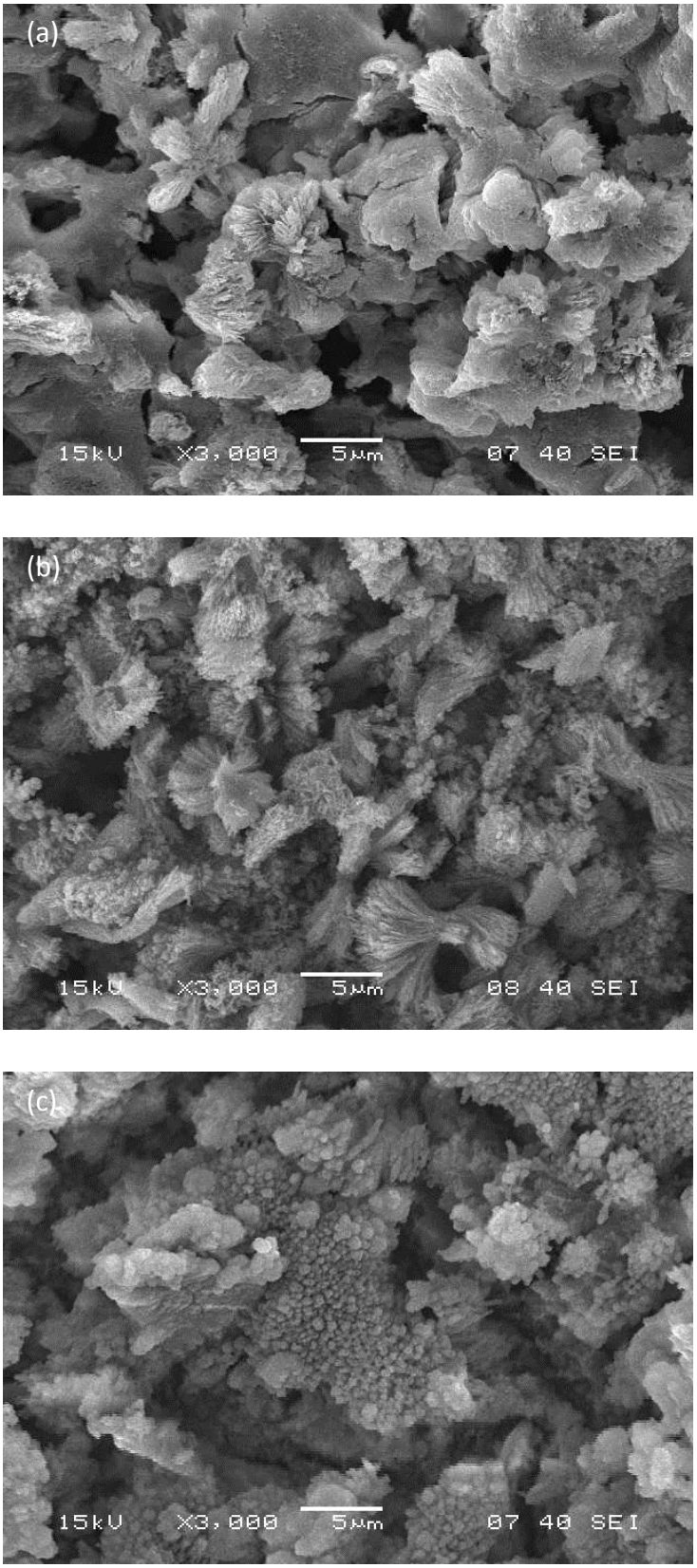


Fig 4.2: Scanning electron micrograph (3000x magnification) of electrophoretically deposited HA on 316L stainless steel at (a) 30 V - 10 mins, (b) 30 V - 15 mins (c) 30 V - 20 mins

Figures 4.2 (a), (b) and (c) show the scanning electron micrograph of HA coated 316L stainless steel sample for three different time durations (10, 15 and 20 mins respectively) at 30 Volts. From the figures, it is observed that HA is deposited in a feathery form with good coverage over the entire surface. The coating thus formed on both the samples is quite uniform. The growth of the HA crystals is more pronounced in the 20 mins sample as compared to the 10 and 15 mins samples, which leads to better coating on the surface as observed from the figures. This better coverage of the coating in the 20 mins treated sample is due to the fact that a proper growth of the HA crystals has taken place with more time, which also results in giving a good thick layer on the surface.

4.2.1.2. Analysis of pore structure

Figure 4.3 shows the distribution of pores of the HA coating on 316L SS with time. Fig 4.3 (a) and (b) show that the pore size decreases with an increase in the coating time. The average pore size (calculated on the basis of simple mathematical averages) decreases from 316 nm to 253 nm.

This is because of the fact that growth of the HA crystals occurs with greater deposition times and the crystals cover up maximum area on the implant surface leaving very small holes.

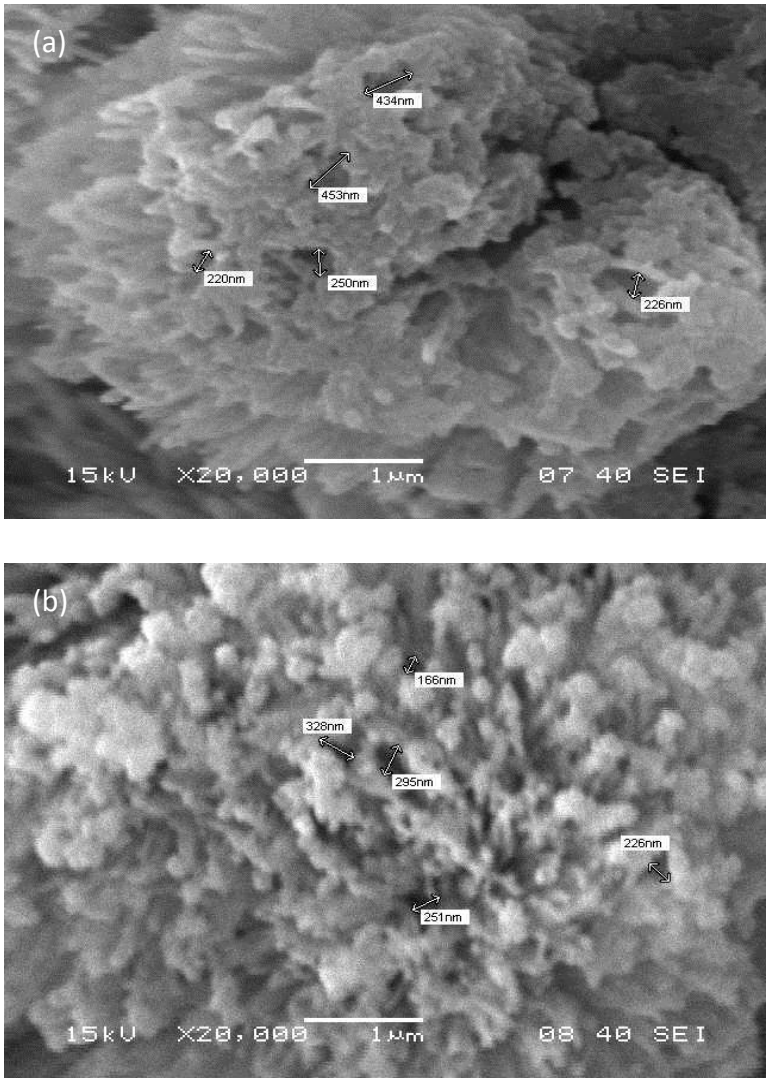


Fig 4.3: Scanning electron micrograph of electrophoretically deposited HA on 316L stainless steel at (a) 30 V - 10 mins (b) 30 V - 15 mins

4.2.2. Phase Analysis

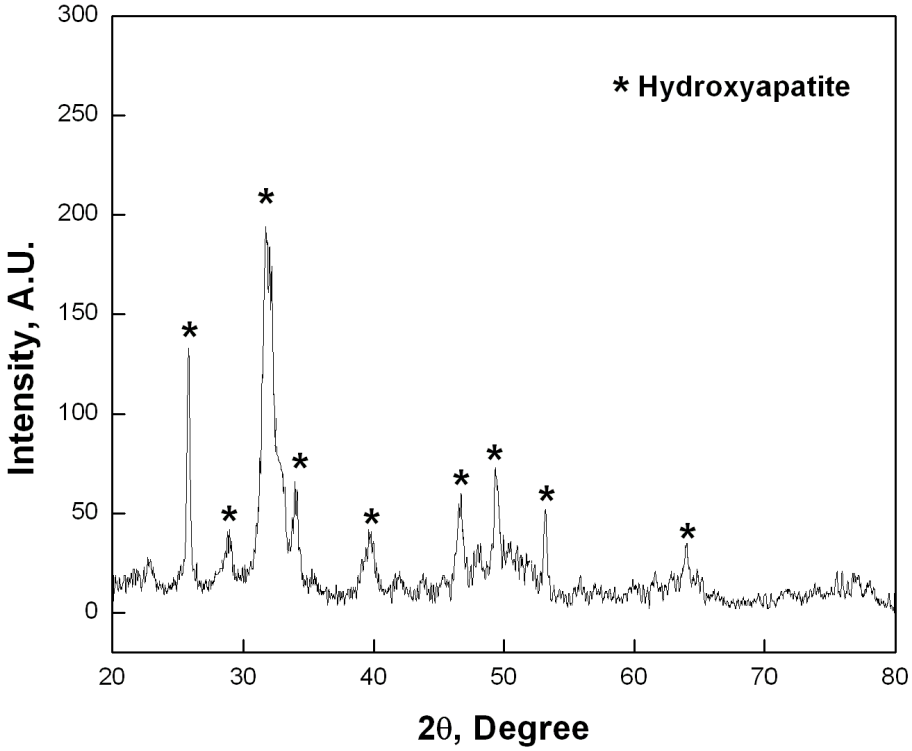


Fig 4.4: Scanning electron micrograph of electrophoretically deposited HA on 316L stainless steel at (a) 30 V - 10 mins (b) 30 V - 15 mins

Fig 4.4: shows the X ray diffraction profile of electrophoretically deposited HA on 316L SS at 30 Volts and 10 mins. From the analysis of the XRD data obtained from the scrape of the coating on the sample surface, it was found that that the peaks obtained are a match with the standard peaks of HA in the JCPDS data book. The matching of the peaks are done in PHILIPS X pert high score software. Hence we can conclude that HA has not lost its phase purity after deposition.

The coating thus formed consists of mainly HA as verified from the X Ray Diffraction pattern. No other lower calcium compounds are detected in the data, which confirms that the purity of the coating in terms of HA is almost 99 %.

4.3 Electrophoretic deposition of hydroxyapatite-chitosan composite on 316L stainless steel

4.3.1 Characteristics of the HA-chitosan layer on 316L Stainless steel

4.3.1.1 Microstructural analysis

Figures 4.5 (a), (b) and (c) show the scanning electron micrograph of HA-chitosan composite coated on 316L stainless steel sample for three different applied voltages (20, 25 and 30 volts respectively) for 25 mins. From the figures, it is observed that HA-chitosan is deposited with good coverage over the entire surface without any cracks on it. The crystals of HA look like facets with random orientation on the surface. The microstructure of the coating is finer at higher voltages due to higher driving force at 30 volt compared to 20 volt.

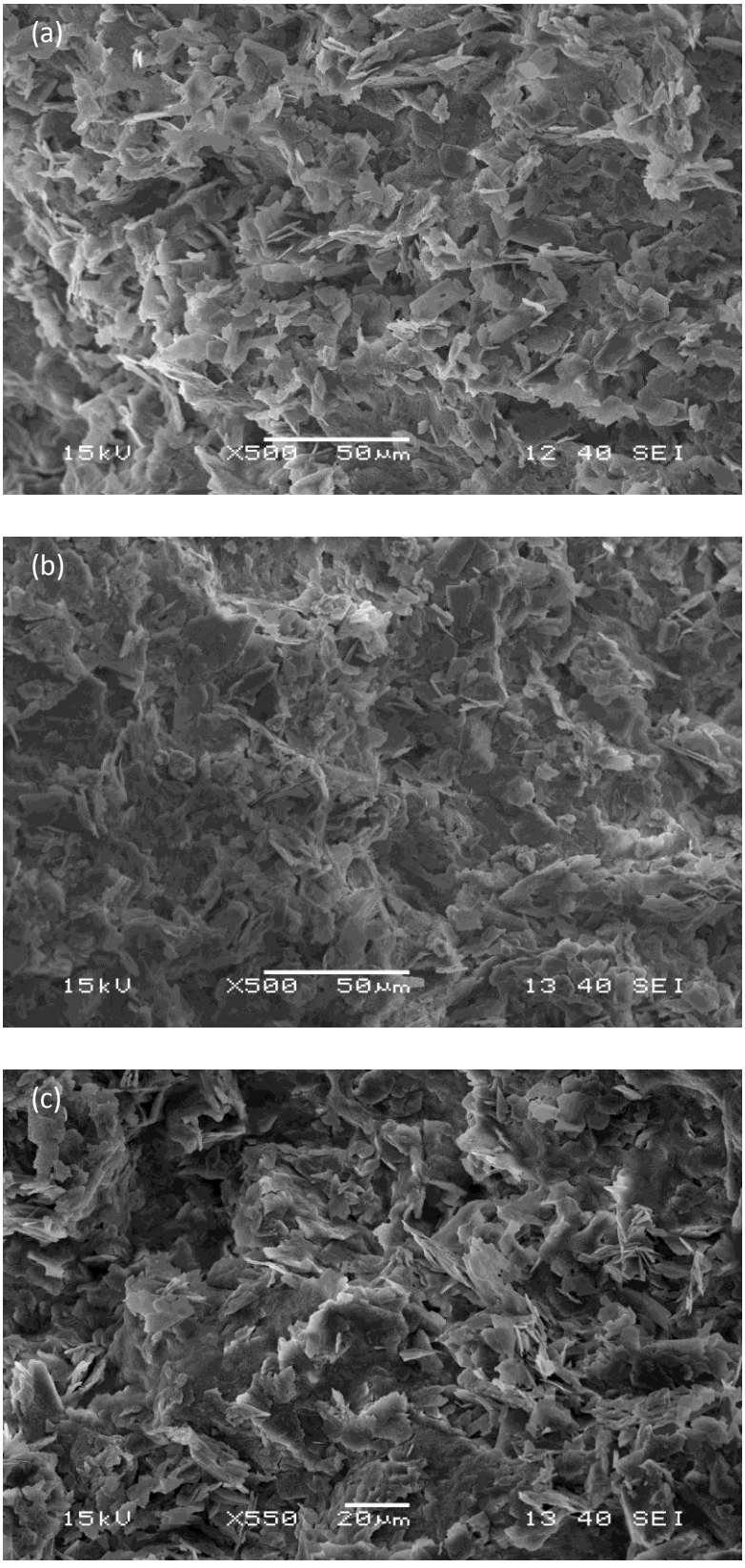


Fig 4.5: Scanning electron micrograph (500x magnification) of electrophoretically deposited HA-chitosan composite on 316L stainless steel at (a) 20 V - 25 mins (b) 25 V - 25 mins (c) 30 V - 25 mins

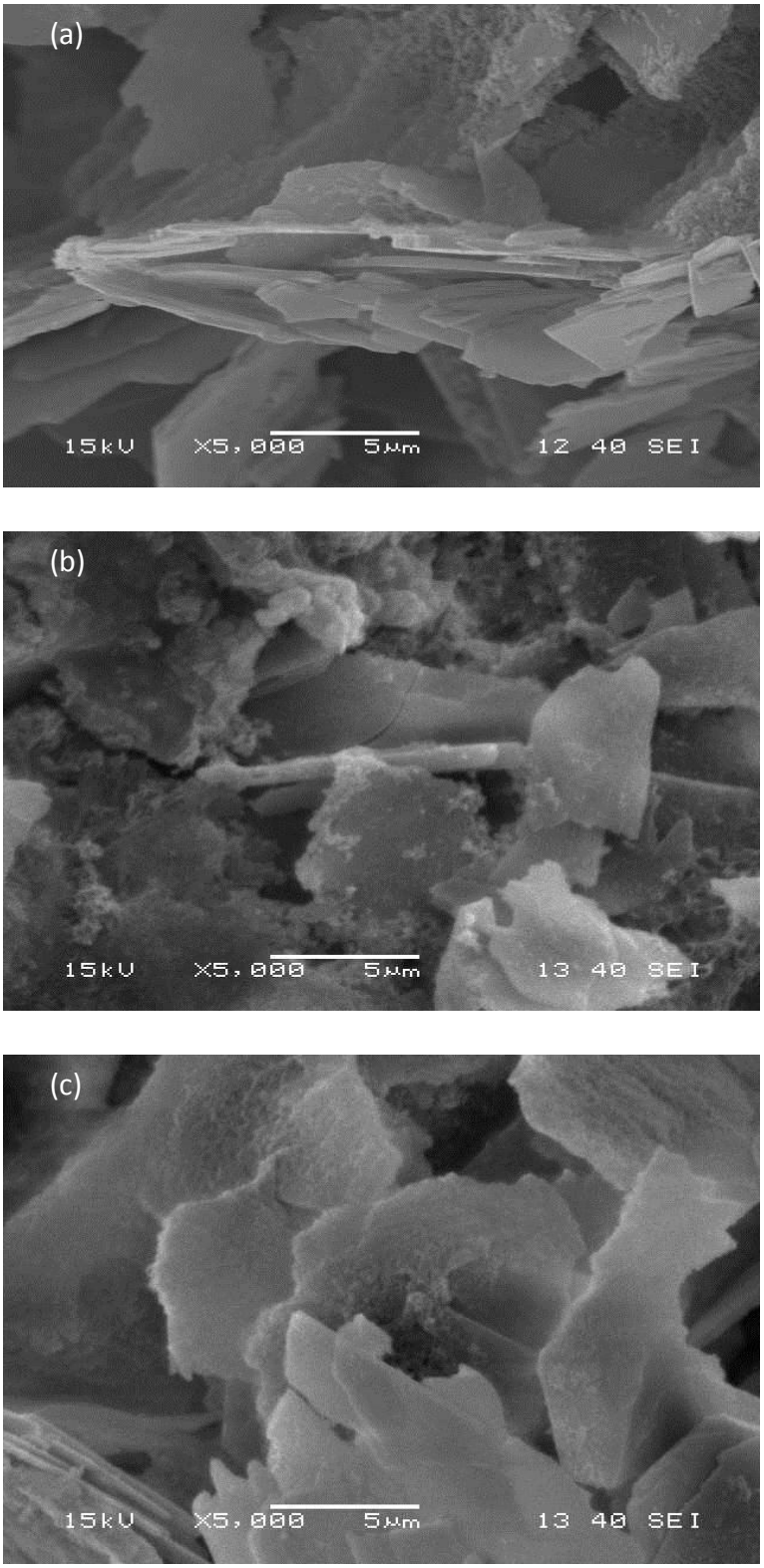


Fig 4.6: Scanning electron micrograph (5000x magnification) of electrophoretically deposited HA-chitosan composite on 316L stainless steel at (a) 20 V - 25 mins (b) 25 V - 25 mins (c) 30 V - 25 mins

From figures 4.6 (a), (b) and (c), it is observed that the coarseness of the coating gets reduced with rise in voltage. This is because at a constant deposition time, with increase in the applied potential difference, there is more nucleation of HA-chitosan composite crystals on the stainless steel surface leading to the formation of finer coatings.

4.3.1.2. Analysis of pore structure

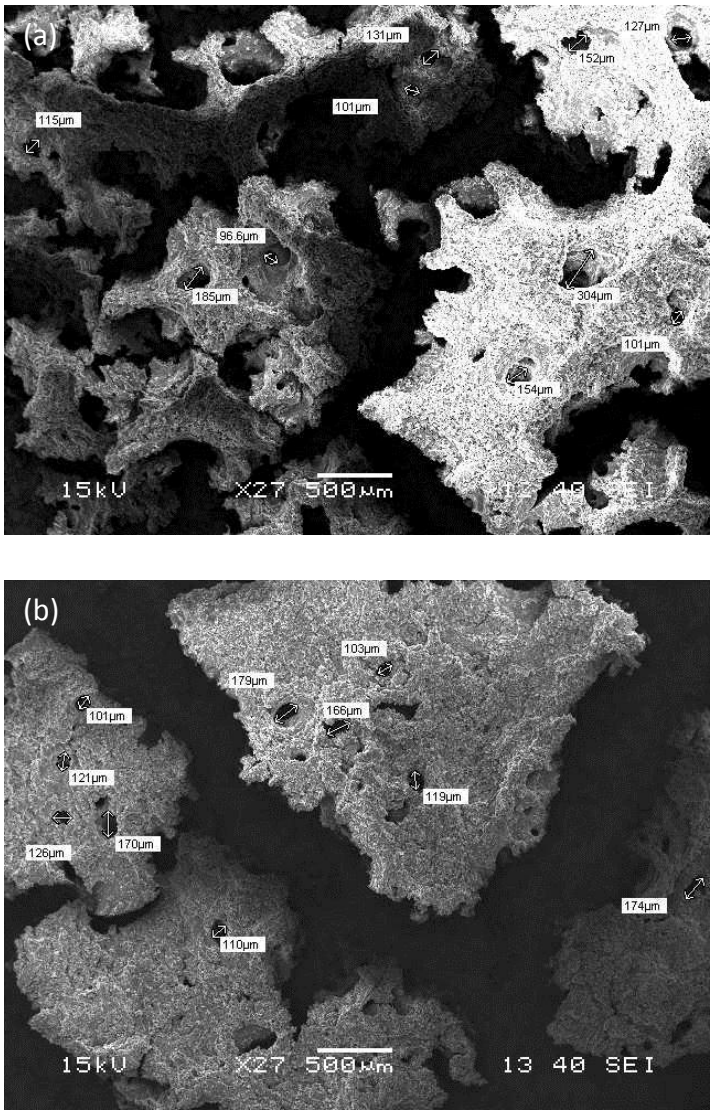


Fig 4.7: Scanning electron micrograph of electrophoretically deposited HA-chitosan composite on 316L stainless steel at (a) 20 V - 25 mins (b) 30 V - 25 mins

With increase in voltage, there is a slight decrease in the size of the pores formed. The average pore size decreases from 146.5 µm to 137 µm. The coating was found to be composed of tiny hydroxyapatite crystals incorporated in the continuous chitosan film.

4.3.2. Phase Analysis

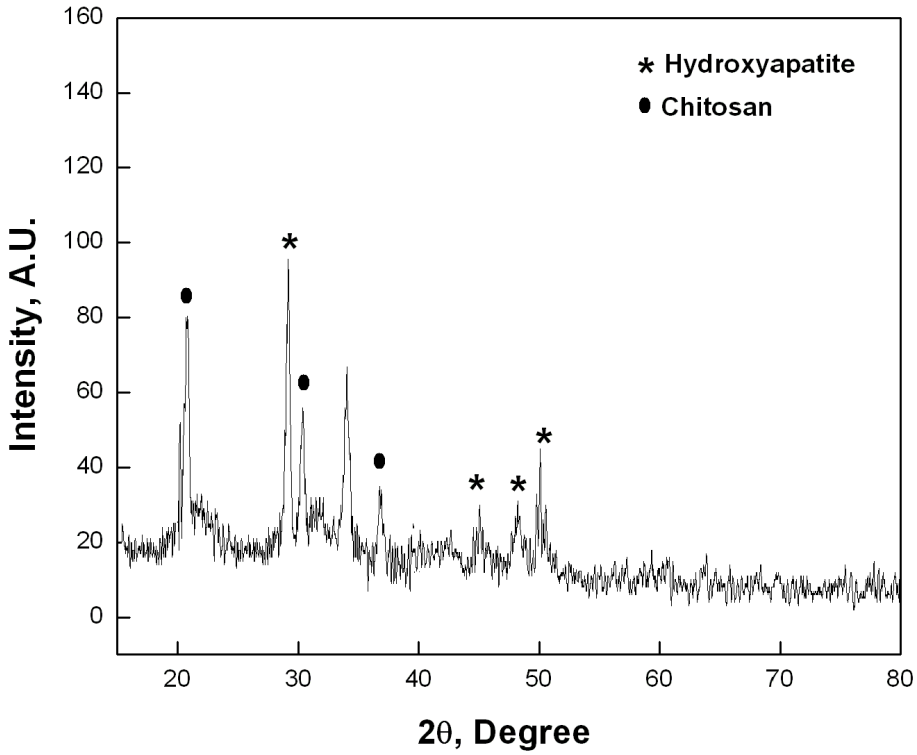


Fig 4.8: Scanning electron micrograph of electrophoretically deposited HA-chitosan composite on 316L stainless steel at (a) 30 V - 10 mins (b) 30 V - 15 mins

From the analysis of the XRD data obtained from the sample surface, it was found that that the peaks obtained match with the standard peaks of HA and chitosan in the JCPDS data book. This is done by PHILIPS X pert high score software.

Hence we can conclude that neither HA nor chitosan lose their phase purity upon deposition and the presence of both in the coating is verified from the XRD plot.

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CONCLUSION

1. HA and HA-chitosan were successfully coated onto 316L SS at lower voltages.

2. Electrophoretic deposition gives proper coating thickness, and the phase purity of HA and HA-chitosan was maintained after deposition.

3. Since the pore sizes are in the range of 50-320 nm, the coatings are suitable for cell attachment and proliferation.

4. With increase in voltage, there is more nucleation; with increase in time, there is growth of crystals. So a proper combination of voltage and time can effectively coat the steel surface with HA/ HA-Chitosan with desired pore size for bioimplant applications.

5. For HA coating the best coating parameters are found to be 30 volt and 15 mins. With increase in deposition time, there is an increase in both the density and coarseness of the HA coating formed. This happens because with increase in deposition time, the deposited HA crystals get more time to grow on the 316L SS surface.

6. Electrophoretic deposition method can effectively be used for HA and HA-chitosan coating by using the solution having HA in ethanol and chitosan in acetic acid.

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